Related article on page 1975

American Journal of Pathology, Vol. 168, No. 6, June 2006

DOI: 10.2353/ajpath.2006.060270

Commentary

Translating Mighty Mice into Neuromuscular Therapeutics

Is Bigger Muscle Better?

Eric P. Hoffman and Diana Escolar

From the Research Center for Genetic Medicine, Children's National Medical Center, Washington, District of Columbia

There is increasing clinical research interest in skeletal muscle due in part to the rising incidence of morbidities associated with muscle, particularly insulin resistance and type 2 diabetes (inactive and over-fed muscle) as well as aging and frailty (sarcopenia). A promising avenue of drug development is focused on muscle hypertrophy: the rationale being that a larger muscle is healthier and stronger. Molecular targets for drug development include the myostatin pathway and the IGF1/AKT1 pathway, with both preclinical and clinical testing underway in the inherited muscular dystrophies and in other muscle disorders. As research intensifies, the complexity of these networks in both normal physiology and pathophysiology is becoming evident. As described by Parsons et al[1] in this issue of *The American Journal of Pathology*, myostatin blockade in mouse models of inherited muscle disease is highly variable in its effectiveness, depending on the disease model used, the specific muscle groups studied, and even the age of the animal. Here, we discuss the underlying rationale and current data concerning modulation of muscle size in clinical disorders of muscle.

The Physiology of Muscle Remodeling

Skeletal muscle is the largest organ system in the human body, typically comprising about 30% of body mass. As an organ, muscle undergoes extensive remodeling as a function of use (training). With resistance training, muscle groups become larger and stronger, whereas aerobic training leads to metabolic remodeling. Common wisdom suggests that there is an excellent correlation between muscle strength and size. However, the correlations between strength and size are not as good as one might expect. In human studies of leg muscles,[2,3] the correlation (r) between size and strength is about 0.5 to 0.7; however, this correlation is considerably less in non-weight-bearing muscles. For example, a recent large magnetic resonance imaging muscle volume and strength study of arm muscles of college volunteers found a low correlation with an $r = 0.23$ (Figure 1).[4] There are a number of confounding variables in such studies, such as the proportion of fast twitch and slow twitch fibers in the muscle group and the unknown relationship between muscle volume and fiber cross-sectional area.

The relevant clinical parameter of muscle is strength, although muscle also plays a key role in systemic metabolism (eg, insulin resistance). If muscle size is only one-fourth to two-thirds of the strength story, what are some other factors resulting in "strength?" Fiber type distribution is one factor, in that a preponderance of type II fibers is positively correlated with muscle strength. Also, the ability of the central nervous system to activate muscle fibers (motor unit recruitment) is well known to be a key component of strength. Genetics is a surprising large component, likely due to its influence on fiber type distribution, muscle size, the ability to activate motor units, and other contributors. Through twin and family studies, the genetic components of muscle size and strength are quite large, with most estimates of heritability of strength and size hovering around 0.7 (70% genetic vs. 30% environmental).[5,6] Through twin and family studies, the genetic components of muscle size and strength are quite large, with most estimating the heritability of strength and size to hover around 0.7 (70% genetic versus 30% environmental).[5,6]

Thus, muscle strength and size result from complex interactions between the predispositions of genetics (inherited strength, neuronal and motor unit components,

Accepted for publication March 21, 2006.

This commentary relates to Parsons et al, Am J Pathol 2006, 168:1975-1985, published in this issue.

Address reprint requests to Eric P. Hoffman, Research Center for Genetic Medicine, Children's National Medical Center, 111 Michigan Ave. NW, Washington, DC 20010. E-mail: ehoffman@cnmcresearch.org.

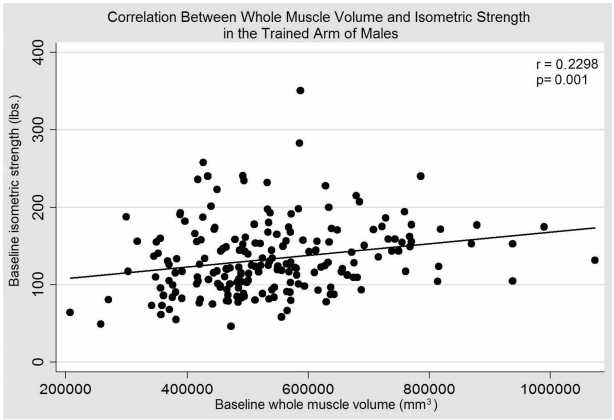

Figure 1. Relatively poor correlation of arm muscle size and strength in young adult males. Shown is data from a study of 1000 university students using volumetric magnetic resonance imaging and isometric strengths measurements (see Ref. [4] for primary data).

and tissue volume) and the environment (training or use patterns, disease states, and aging). With this backdrop, it may not be surprising that Parsons et al[1], in this issue of *The American Journal of Pathology*, show that larger muscle created by myostatin blockade does not necessarily equate to stronger muscle. But before we turn to this important study in detail, we first discuss the normal physiological pathways involved in modulating muscle size and how these pathways are affected in the pathologies of muscle. We then turn to efforts to pharmacologically modulate these pathways to improve muscle function and the new data from Parsons et al.

Hyperplasia, Hypertrophy, and Anti-Atrophy

There are a number of ways that muscle can become larger (hypertrophic), including hyperplasia (increased number of cells), growth of each cell (myofiber hypertrophy), and inhibition of muscle atrophy pathways.

Hyperplasia occurs only during embryonic and fetal development, and it is currently thought to be difficult if not impossible to increase the number of myofibers in a postnatal human (via drugs, or any other means). The basal lamina sheaths that envelope each large syncytial myofiber define that "cellular space" throughout postnatal life. If a fiber is damaged and needs to regenerate, it does so within the pre-existing basal lamina using muscle precursor cells (stem cell progeny) that share the same basal lamina. Hyperplasia during embryonic and fetal development may occur, where one individual may be genetically predisposed to be born with more fibers than another person. A key point here is that transgenic mouse models may show hyperplasia if the transgene is expressed during embryogenesis; however, such success in achieving hyperplasia is not likely relevant to human clinical medicine. In other words, few researchers are currently envisioning preventive medicine that can be practiced through transgenic humans or prenatal gene therapy. We are thus stuck with the number of fibers we are born with, and clinical medicine must work within these constraints.

The growth in size of individual muscle cells, myofiber hypertrophy, can occur with or without the fusion of muscle precursor cells (myoblasts). Little is currently known regarding the extent to which satellite cells (dormant myoblasts) can activate and contribute to hypertrophy of a viable myofiber. There are two well-described biochemical pathways that alter myofiber size: the AKT1 signaling pathway, which responds to both hypertrophic and atrophic stimuli, and the myostatin pathway, which inhibits muscle growth.

The AKT1 pathway has been the focus of a number of recent reviews.[7,8] AKT1 is a key signaling protein that receives a variety of signals from cell membrane receptors and their ligands, such as IGF1 and insulin, which then signal through PI3 kinase to alter the phosphorylation state of AKT1. AKT1 then itself phosphorylates a series of downstream targets, including FOXO transcription factors (activating them and inducing atrophy) and mTOR and GSK3β (both of which lead to increased protein synthesis during hypertrophy). To date, there are no clinical trials underway for modulation of AKT1 activity as a means of promoting muscle strength, although there is considerable pharmaceutical interest in heading in this direction.

The other key atrophy/hypertrophy pathway involves myostatin (GDF8). Myostatin is a member of the TGF-β family of proteins, which is often associated with negative regulation of growth and induction in many pathological settings. Mice and cows with loss-of-function mutations of myostatin show significant muscle hypertrophy due to both developmental hyperplasia and postnatal myofiber hypertrophy.[9,10] Conditional knockouts in which myostatin is lost only in adult muscle show myofiber hypertrophy but not hyperplasia. These data are consistent with myostatin functioning as a negative regulation of muscle growth. A recent study of deacetylase inhibitors demonstrated induction of follistatin, which in turn increased the rate of myoblast fusion leading to increased myotube size.[11] Follistatin is a protein that binds circulating myostatin and inhibits its function. Overexpression of follistatin also leads to very large muscles. Myostatin blockade has become an important avenue for modulating muscle size and hopefully muscle function, and this is the focus of the Parsons et al study in murine models of muscular dystrophy.

Myostatin in Muscle Disease

The Parsons et al[1] study describes the effects of myostatin blockade in mouse models of muscular dystrophy, namely δ-sarcoglycan deficiency. This mouse is genetically and biochemically similar to a very rare type of limb-girdle muscular dystrophy (only two patients in the U.S. have been identified to date); however, δ-sarcoglycan deficiency in humans clinically resembles the more common Duchenne muscular dystrophy (DMD; dystrophin-deficiency). The δ-sarcoglycan mouse model, like the *mdx* mouse model for DMD, shows a staged pathological pattern, with normal muscle up to about 3 weeks of age, followed by a bout of widespread myofiber death

(necrosis) and subsequent "successful" regeneration and then a more chronic degeneration/regeneration pattern afterward. Fifty percent of δ-sarcoglycan mice have been reported to die by 28 weeks of age,[12] and Parsons et al state that this makes the mouse a more valid model for the clinically progressive DMD. This is arguable, because the severity of the mouse models seems to depend on the genetic background of the mice, and the reasons for the death of the δ-sarcoglycan mice are not known (cardiac failure has been proposed). Although the original studies describe early mortality in these mice, Parsons et al study mice out to 38 weeks yet do not mention any difficulty keeping them alive. Thus, the authors' statements that the δ-sarcoglycan model used is "more similar" to DMD is open to considerable debate.

Model aside, the Parsons et al study is the most extensive publication to date regarding the applications of myostatin blockade to any neuromuscular disease. The authors did an outstanding job of covering many different variables. They used multiple methods of myostatin blockade (neutralizing antibodies administered intravenously or genetic removal of myostatin by crossing the myostatin null [knock out] strain with the muscular dystrophy model). In addition, they studied multiple functional and histological endpoints, including muscle size and strength, quantitative histology, and cell morphometry. Most importantly, the authors studied two age ranges (young, 4 weeks; older, 20 to 36 weeks) and also assayed an impressive array of different muscle groups.

The key finding of the study is that myostatin blockade is highly variable in its effectiveness, with some muscle groups showing a doubling of muscle size (eg, tibialis anterior with treatment at 4 weeks) and others showing significant decreases in muscle size (gastrocnemius with treatment at 20 weeks). The authors normalize the muscle weights to total body weight so that the absolute size increase of each muscle is not obvious. Other histological markers also showed variable response, with some decrease in fibrosis, and evidence for an increase in regeneration. In general, younger mice seemed to do better with myostatin blockade than did older mice.

The authors also tested muscle strength using front paw grip strength, but there was little evidence of any increase in strength. This measurement is considered relatively crude and did not functionally test the hindlimb muscles, which were studied histologically. Future studies should be focused on ex vivo functional tests of a variety of muscle groups followed by correlation of muscle size, histology, and resulting functional changes.

This report adds considerable new data to what is emerging as a very complex story. Two other mouse models of muscular dystrophy have been studied with regard to myostatin blockade: the dystrophin-deficient *mdx* mice (a model for DMD) and the laminin α2-deficient *dy/dy* mice (a model for severe congenital muscular dystrophy). In the relatively young and clinically mild *mdx* mouse, myostatin blockade showed significant improvement in size and strength.[13,14] On the other hand, block of myostatin by use of follistatin in the very severe *dy/dy* mice led to smaller muscles and an earlier death.[15] The new data by Parsons et al[1] on the δ-sarcoglycan-deficient mice are consistent with the inconsistencies of previous studies, with myostatin blockade beneficial to young dystrophic mice but deleterious to older, more clinically involved mice. However, even more importantly, Parsons et al provide the first documentation of the considerable muscle-to-muscle variability in response.

In summary, the Parsons et al work provides the most extensive studies of myostatin blockade in animal models of neuromuscular disease to date. Their results suggest that the effects of myostatin blockade are highly variable, depending on the specific muscle group examined as well as the age of the mouse. Overall, myostatin inhibition appears to be a good method to increase muscle size, but the effects on improving muscle function remain quite cloudy. Toward this end, applications to human neuromuscular conditions may require careful selection of subjects, with focused studies of the effects of the treatment on different muscle groups. Finally, it is pertinent to note that muscle from DMD patients shows extensive repression of myostatin as part of the disease pathophysiology.[16] Thus, myostatin blockade may be expected to have less of an effect in DMD than it would have in aging muscle where endogenous myostatin levels are presumably higher.

Future studies may screen for drugs that directly influence muscle strength, rather than using muscle size as a surrogate marker for strength. Genetic studies that identify "strength genes" may point to appropriate biochemical pathways for pharmacological intervention for strength itself, just as mighty mice and double-muscled cattle have pointed to the myostatin pathway for muscle size.

Acknowledgments

The authors thank Priscilla Clarkson and Terence Partridge for comments on the manuscript.

References

1. Parsons SA, Millay DP, Sargent MA, Mitchell R, McNally EM, Crandall DL, Molkentin JD: Age-dependent effect of myostatin blockage on disease severity in a murine model of limb-girdle muscular dystrophy. Am J Pathol 2006, 168:1975–1985
2. Trappe SW, Trappe TA, Lee GA, Costill DL: Calf muscle strength in humans. Int J Sports Med 2001, 22:186–191
3. Masuda K, Kikuhara N, Takahashi H, Yamanaka K: The relationship between muscle cross-sectional area and strength in various isokinetic movements among soccer players. J Sports Sci 2003, 21:851–858
4. Hubal MJ, Gordish-Dressman H, Thompson PD, Price TB, Hoffman EP, Angelopoulos TJ, Gordon PM, Moyna NM, Pescatello LS, Visich PS, Zoeller RF, Seip RL, Clarkson PM: Variability in muscle size and strength gain after unilateral resistance training. Med Sci Sports Exerc 2005, 37:964–972
5. Perusse L, Lortie G, Leblanc C, Tremblay A, Theriault G, Bouchard C: Genetic and environmental sources of variation in physical fitness. Ann Hum Biol 1987, 14:425–434
6. Zhai G, Ding C, Stankovich J, Cicuttini F, Jones G: The genetic contribution to longitudinal changes in knee structure and muscle strength: a sibpair study. Arthritis Rheum 2005, 52:2830–2834

7. Hoffman EP, Nader GA: Balancing muscle hypertrophy and atrophy. Nat Med 2004, 10:584–585
8. Glass DJ: Signaling pathways that mediate skeletal muscle hypertrophy and atrophy. Nat Cell Biol 2003, 5:87–90
9. Grobet L, Martin LJ, Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Menissier F, Massabanda J, Fries R, Hanset R, Georges M: A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nat Genet 1997, 17:71–74
10. Szabo G, Dallmann G, Muller G, Patthy L, Soller M, Varga L: A deletion in the myostatin gene causes the compact (Cmpt) hypermuscular mutation in mice. Mamm Genome 1998, 9:671–672
11. Iezzi S, Di Padova M, Serra C, Caretti G, Simone C, Maklan E, MinettiG, Zhao P, Hoffman EP, Puri PL, Sartorelli V: Deacetylase inhibitors increase muscle cell size by promoting myoblast recruitment and fusion through induction of follistatin. Dev Cell 2004, 6:673–684
12. Hack AA, Lam MY, Cordier L, Shoturma DI, Ly CT, Hadhazy MA, Hadhazy MR, Sweeney HL, McNally EM: Differential requirement for individual sarcoglycans and dystrophin in the assembly and function of the dystrophin/glycoprotein complex. J Cell Sci 2000, 113:2535–2544
13. Wagner KR, McPherron AC, Winik N, Lee SJ: Loss of myostatin attenuates severity of muscular dystrophy in mdx mice. Ann Neurol 2002, 52:832–836
14. Bogdanovich S, Krag TO, Barton ER, Morris LD, Whittemore LA, Ahima RS, Khurana TS: Functional improvement of dystrophic muscle by myostatin blockade. Nature 2002, 420:418–421
15. Li ZF, Shelton GD, Engvall E: Elimination of myostatin does not combat muscular dystrophy in dy mice but increases postnatal lethality. Am J Pathol 2005, 166:491–497
16. Chen YW, Nagaraju K, Bakay M, McIntyre O, Rawat R, Shi R, Hoffman EP: Early onset of inflammation and later involvement of TGFbeta in Duchenne muscular dystrophy. Neurology 2005, 65:826–834